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Root Development: The Embryo Within?

Robert Sablowski

Root growth is sustained by stem cells maintained at the root tip. Recent evidence suggests that genes required to maintain root stem cells also specify root identity in the early embryo.

In both plants and animals, stem cells divide regularly to supply undifferentiated cells for the formation of new tissues. The stem cells are typically maintained by an intercellular signal that acts with a limited range. The specialised cells that produce this signal control the location and number of stem cells maintained within the tissue. A recent paper from the Scheres lab [1] has identified genes required to establish the source of the maintenance signal for root stem cells in *Arabidopsis* and suggests that the same genes function early in embryogenesis to specify root identity.

Root growth is sustained by cell division within a meristem located at the root tip [2]. At the core of the meristem, stem cells produce new cells that accumulate in regular files. The cells left behind by the growing tip differentiate to form the body of the root, while cell files that accumulate ahead of the tip differentiate as the root cap, which protects the meristem and whose cells are regularly shed as the root grows through the soil. The root stem cells surround a small group of cells called the quiescent centre (Figure 1C). The quiescent centre is the source of a signal that maintains the stem cells: if the quiescent centre is eliminated, either by laser ablation or by mutation, the stem cells differentiate and root growth stops [3,4]. Quiescent centre cells are thus central for determining where and for how long root tips can grow.

How is the quiescent centre positioned? Previous work has shown that the quiescent centre is established at the overlap of two inputs. One is the regulatory gene *SCARECROW* (*SCR*), which functions in a cup-shaped area within the root tip, including the quiescent centre and the cells that give rise to the cortex and endodermis of the root [4]. The other is the phytohormone auxin, which is continuously transported to the root tip [5]. Auxin transport to the basal part of the plant starts early in embryogenesis and is required for development of the embryonic root [6]. Later in life, auxin levels remain high in the vicinity of the quiescent centre, and it has been proposed that the quiescent centre is specified where *SCR* is combined with high levels of auxin [4].

Aida *et al.* [1] identified the *PLETHORA* (*PLT*) genes as mediators of the auxin input to establishment of the quiescent centre. *PLT1* and *PLT2* encode closely related proteins of the AP2 family of transcription factors. In the *plt1*, *plt2* double mutant — from hereon referred to as the *plt* mutant for simplicity — the root stem cells showed signs of differentiation, such as accumulation of starch grains. With reduced stem cell activity, the population of dividing cells in the meristem dwindled and root growth eventually stopped.

The loss of stem cell activity in the *plt* mutant was traced to a failure to specify quiescent centre cells. This was shown by the lack of expression of quiescent centre marker genes, seen from the early stages of embryogenesis, when the quiescent centre is first established. The expression pattern of *PLT* genes was also consistent with a role in specifying the quiescent centre: the initial expression was seen in the basal half of eight-cell stage embryos (Figure 1B) and later included the cells that give rise to the quiescent centre. In the mature meristem, *PLT* expression was seen to remain high in the quiescent centre and in the surrounding cells.

PLT genes function in parallel with *SCR* and downstream of auxin to establish the quiescent centre. Expression of *SCR* was normal in the *plt* mutant, while *PLT* expression was unaffected in the *scr* mutant; when the *plt* and *scr* mutations were combined, the meristem terminated even earlier, showing that *SCR* was still active in the *plt* mutant background. *PLT* genes were induced by external auxin treatment and repressed by a dominant mutation (*iaa14*) that inhibits responses to auxin. Mutations that disrupt the effect of auxin in root establishment, *mp* and *nph4*, also diminished *PLT* expression. Consistent with the *PLT* genes acting downstream of auxin, external auxin treatment did not rescue the quiescent centre in the *plt* mutant.

The overall conclusion from these experiments was that *PLT* genes mediate the effect of auxin in positioning the quiescent centre. A surprising twist, however, came from experiments in which *PLT* was expressed throughout the embryo and found to cause various degrees of conversion of shoot into root tissues [1]. In severe cases, the shoot apex had foci with ectopic expression of quiescent centre markers and was covered in cells resembling those at the root cap. Before this, the only example of transformation of embryonic shoot into roots that had been reported was the temperature-sensitive *topless-1* (*tpl-1*) mutant [7]. The *tpl-1* mutation affects a putative transcriptional co-repressor (Jeff Long, personal communication), raising the possibility that the ectopic roots seen in the mutant might be caused by derepression of *PLT* genes.

The dual role of *PLT* in maintaining root stem cells and in specifying root identity mirrors recent work on the shoot stem cells. In the shoot, the *WUSCHEL* (*WUS*) gene specifies the organising centre, which produces a signal that maintains the neighbouring shoot stem cells and thus has a role analogous to that of the quiescent centre [2] (Figure 1C). When *WUS*

Cell and Developmental Biology, John Innes Centre, Norwich NR4 7UH, UK. E-mail: Robert.Sablowski@bbsrc.ac.uk

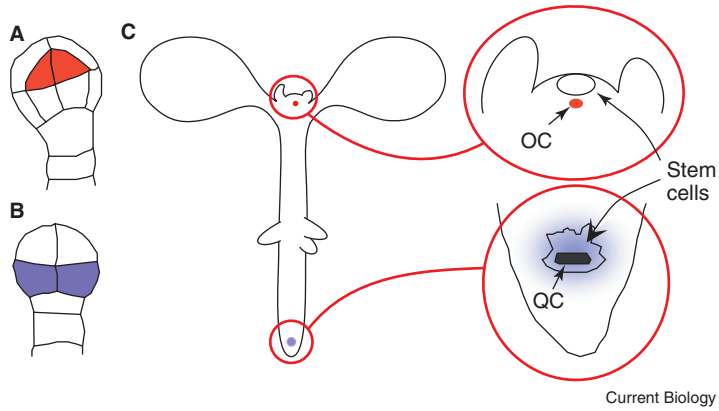

Figure 1. A schematic view of the expression patterns of genes required to maintain stem cells in *Arabidopsis*.

(A) Sixteen-cell embryo, showing the earliest detectable expression of *WUS* (red), within the apical half of the embryo [9]. (B) Expression of *PLT* (blue) is first seen at a slightly earlier stage (eight-cell embryo), in the basal half of the embryo [1]. (C) In the seedling, expression of *WUS* (red) and *PLT* (blue) is restricted to small groups of cells within the shoot and root tips, shown at higher magnification on the right. Shoot stem cells overlie the organising centre (OC, red), while root stem cells surround the quiescent centre (QC, hatched). *WUS* (red) is expressed in the organising centre [9] and *PLT* is expressed in the organising centre and neighbouring cells, including the stem cells [1].

was ectopically expressed in the root meristem, neighbouring cells expressed a marker for shoot stem cells and went on to make shoot tissues, suggesting that *WUS* not only establishes stem cells, but also specifies their shoot identity [8]. This function would be consistent with the early expression of *WUS* within the region of the embryo that gives rise to the shoot [9] (Figure 1A).

So, in both the root and the shoot, genes required for stem cell maintenance may also have a role in specifying root and shoot identity, respectively. One caveat, however, is that *wus* and *plt* mutants still develop recognisable, even if defective, shoots and roots. Although these genes are expressed very early in embryogenesis, the mutant phenotypes are only detectable at later stages, during meristem development [1,9]. On the other hand, earlier functions may be covered by redundancy. In the case of *WUS*, expression of at least one close homologue overlaps with *WUS* in the early embryo [10], whereas in the case of *PLT*, RNA interference (RNAi) lines were found to lack roots, suggesting that additional homologs function redundantly with *PLT* to specify the root [1].

PLT and *WUS* are among several regulatory genes that are first expressed in the embryo and continue to function in the meristems throughout the plant's life [11]. The expression in embryos and meristems might be a trivial consequence of the fact that much of embryogenesis is concerned with establishing the meristems. What makes *PLT* and *WUS* remarkable, however, is their very early expression, preceding any visible meristem development, and their potential to confer root and shoot identity. Thus, genes responsible for maintaining the stem cell populations in plants may have a role in the initial steps of embryo patterning, in line with the idea that stem cells represent an embryonic state that is retained — or in some cases, reactivated — later in life [12]. To test to what extent plant stem cells share features with early embryonic cells, it will be necessary to look beyond a few regulatory genes and compare global gene expression in these cells. This may be facilitated by recent advances in cell-specific transcriptional profiling [13].

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